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Tyramine fragment binding to BACE-1

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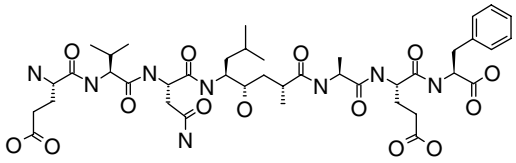
Abstract—Fragment screening revealed that tyramine binds to the active site of the Alzheimer's disease drug target BACE-1. Hit expansion by selection of compounds from the Roche compound library identified tyramine derivatives with improved binding affinities as monitored by surface plasmon resonance. X-ray structures show that the amine of the tyramine fragment hydrogen-bonds to the catalytic water molecule. Structure-guided ligand design led to the synthesis of further low molecular weight compounds that are starting points for chemical leads.

The most prominent neuropathological finding in Alzheimer's disease brains is the occurrence of cortical plaques containing β-amyloid peptide. Genetic evidence points to a causal role of β-amyloid during development of Alzheimer's disease as it is postulated by the amyloid cascade hypothesis.[1] Several independent approaches led to the identification of β-site amyloid precursor protein cleavage enzyme 1 (BACE-1) as the first enzyme of the amyloid cascade. BACE-1 cleaves the 695 or 751 amino acid amyloid precursor protein to a 99 amino acid long precursor peptide which is later trimmed by γ-secretase to form the pathological 40 or 42 amino acid long β-amyloid peptide. BACE-1 is an integral membrane protein with high homology to other aspartic proteases. The enzymatic activity does not depend on the presence of the transmembrane or intracellular domains. Given the lack of adverse effects in gene knockout mice BACE-1 is considered a prime target for the development of Alzheimer's disease therapeutics.[2]

BACE-1 possesses a bilobal structure[3] typical for eukaryotic aspartic proteases[4] with the catalytic aspartate residues D32 and D228 located in the substrate binding cleft between the N-terminal and C-terminal lobes.[3] The active site is partially covered by a flexible hairpin loop referred to as the 'flap'. In apo structures of BACE-1, the flap backbone is about 12 Å away from the side chains of D32 and D228 which coordinate the catalytic water molecule,[5,6] whereas in complexes with peptidomimetic inhibitors like OM99-2 (Fig. 1) the flap moves about 4 Å closer to the catalytic center and the non-cleavable transition state isostere moiety of the inhibitors replaces the catalytic water molecule.[3,7] The only reported exception is an oxyacetamide inhibitor that forms a hydrogen bond to the catalytic water rather than replacing it.[8]

To exert their pharmacological activity in vivo, BACE-1 inhibitors have to traverse the blood–brain barrier adding another level of complexity for drug discovery. In order to identify new lead molecules of low molecular weight, we have applied a fragment screening strategy.[9] Using a combination of computational chemistry and surface plasmon resonance measurements, we selected 48 compounds of molecular weight 100–150 Da for soaking into BACE-1 crystals and subsequent X-ray



7, K_D = 0.04μM, LE= 0.16

Figure 1. OM99-2[3] with BACE-1 K_D value determined by surface plasmon resonance and calculated ligand efficiency (LE).

Keywords: BACE-1; Alzheimer; Fragment screening; Surface plasmon resonance; X-ray structure; Structure-based design; Tyramine.
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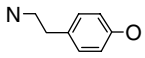
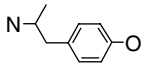
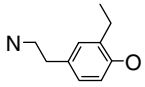
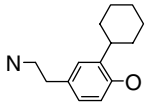
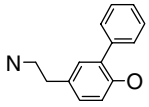
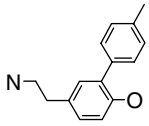
structure determination. Compounds for which the binding mode could be identified were used as starting point for the design of low molecular weight inhibitors of BACE-1. This is in contrast to the recently reported approach where X-ray crystallography was used without a prior binding assay to identify fragments that bind to BACE-1.[10]

One of the compounds for which a crystal structure was determined is the tyrosine metabolite tyramine **1** (Table 1). It binds to the S1 pocket of the BACE-1 active site (Fig. 2) with K_D = 2000 μM. The flap adopts an open conformation (Fig. 3) as described for the structure of the BACE-1 apo form.[6] The most striking feature of the tyramine **1** binding mode is that the catalytic water molecule remains in the position it adopts in the apo structure. It is situated in an almost symmetric environment between the two carboxylate side chains of D32 and D228 and forms a hydrogen bond with the primary amine of tyramine **1** (Fig. 2). This amine group forms two further hydrogen bonds, a direct one with the carboxylate oxygen of D32 closest to the flap and a water-mediated one with the hydroxyl group of the flap residue Y71, thus satisfying all three protons on the protonated amine group. The hydroxyl group of **1** forms a hydrogen bond with the backbone carbonyl of F108. The tyramine phenylethyl moiety forms a number of rather loose lipophilic interactions with aromatic and aliphatic side chains in the S1/S3 pocket, the strongest of which is presumably an edge-to-face contact to F108. Weaker interactions are formed with L30, Y71, and W115 (shortest contacts ~4 Å). The compound barely reaches the side chain of I110 forming the distal end of the S3 pocket (~5 Å). The ligand efficiency as defined by binding energy per non-hydrogen atom[11,12] of **1** is 0.37 and therefore much higher than the ligand efficiency of peptidomimetic BACE-1 inhibitors like OM99-2[3] which is 0.16 (Fig. 1).

Table 1. Tyramine **1**, its derivatives **2**–**6** with BACE K_D values determined by surface plasmon resonance and calculated ligand efficiency (LE)

	Compound	BACE-1 K_D (μM)	LE
1		2000	0.37
2		800	0.38
3		660	0.36
4		220	0.31
5		350	0.29
6		60	0.35

To expand around the primary screening hit tyramine **1**, 32 compounds containing the tyramine substructure were selected from the Roche compound collection for soaking into BACE-1 crystals and subsequent X-ray structure determination. Here we present the crystal structures of the tyramine derivatives **2**–**4** (Table 1) bound to the active site of BACE-1 (Fig. 2). Compound **2** was used as a racemic mixture. Analysis of difference Fourier omit maps shows that its *R* enantiomer is bound to the protein. The positions of the amine and hydroxyl groups of **1** and **2** and their interactions in the binding pocket are essentially identical in both structures. However, the connecting phenyl-alkyl moieties differ significantly in their positions. In compound **2**, the phenol substructure common to both compounds is rotated by about 15° toward Y71 with the hydroxyl oxygen as pivot point. While the benzylic methylene of **1** is in contact with the L30 side chain, the equivalent moiety of **2** points up toward the flap. The additional methyl group of compound **2** makes hydrophobic interactions both with G230 and the side chain of L30.

The *ortho*-substituted tyramine derivative **3** maintains the position of the amino group and its interactions with the catalytic water and D228 as observed for **1** and **2**, but its phenylethyl moiety is shifted toward the S3 pocket (Fig. 2). The ethyl substituent of **3** reaches into the S3 pocket where it is in contact with the lipophilic side chain of I110 and the backbone carbonyl of Q12, slightly increasing the affinity relative to **1**.

Tyramine derivative **4** protrudes even further into the S3 pocket. Its cyclohexyl moiety interacts with backbone atoms of G11, Q12, G13, and G230, and with the side chains of I110 and T232. These interactions result in a nine-fold affinity increase relative to **1**. While **1**–**3** show ligand efficiencies of 0.36–0.38, **4** exhibits a lower value because of its increased molecular weight.

We reasoned that replacing the cyclohexyl ring of **4** with an aryl ring should lead to compounds of higher affinity. The aryl ring should be situated in the S3 pocket facing the terminal methyl group (CD1) of the I110 side chain at a distance of about 3.7 Å. Indeed, we found that the aryl-substituted compound **5** (K_D = 350 μM) retains the potency of **4**.

Tyramine derivative **6** was made to further probe possible interactions with the S3 pocket (see Supplementry Materials for synthesis methods). **6** was found to be 30 times more potent in the surface plasmon resonance binding assay than the initial fragment tyramine **1** resulting in similar ligand efficiency. This was unexpected, since the modeled binding modes indicated that a para-substituent would interfere with the rim of the S3 pocket (G11). We must therefore assume that there is a

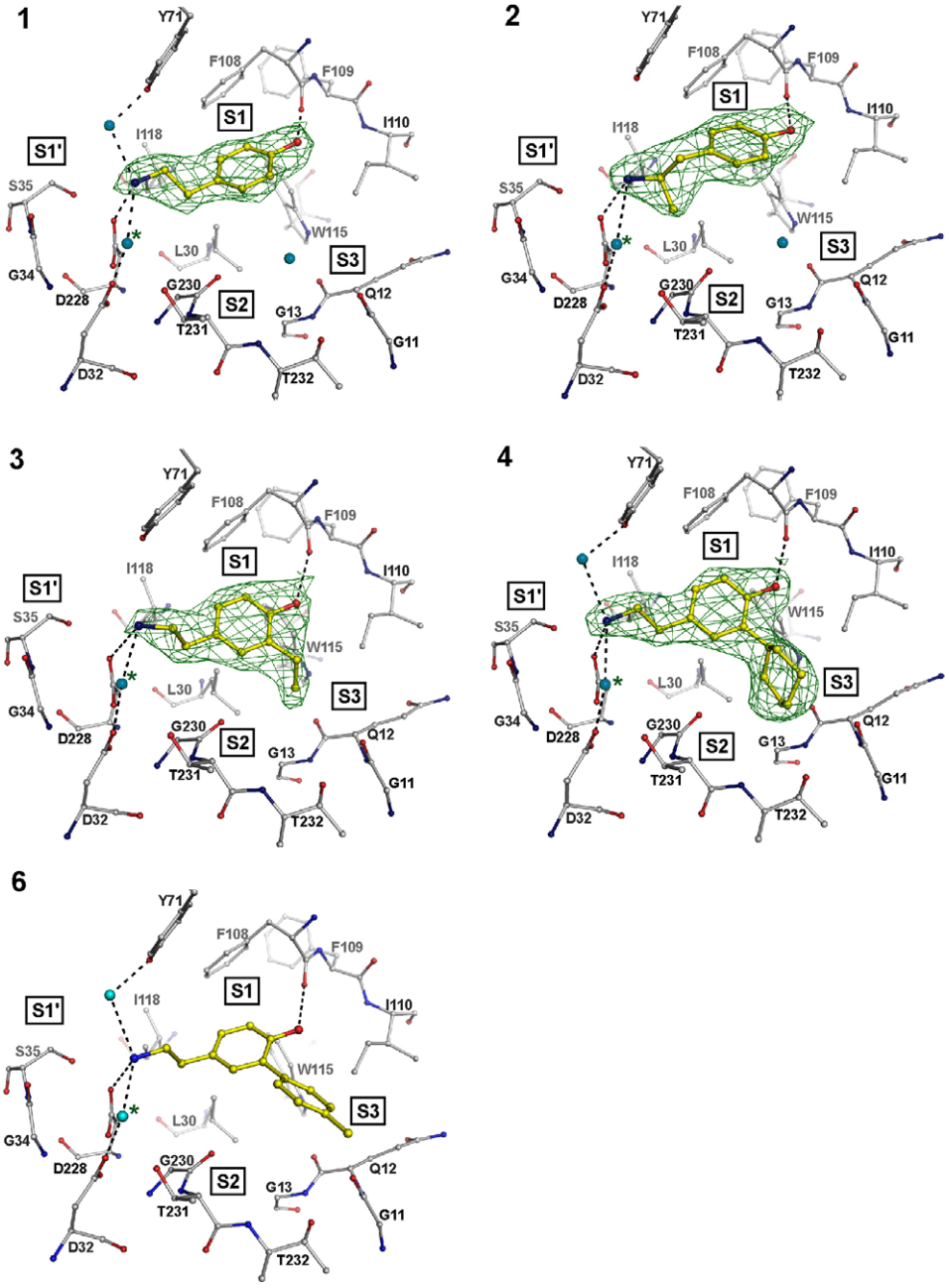


Figure 2. Crystal structures of compounds **1** (2.3 Å resolution, PDB Accession code 3BRA), **2** (2.3 Å, 3BUF), **3** (2.5 Å, 3BUG), and **4** (2.3 Å, 3BUH) bound to the BACE-1 active site.[13] The catalytic water is marked by an asterisk. Fo-Fc omit maps contoured at three standard deviations above the mean density of the map are in green. The binding mode of compound **6** was modeled based on the structural information.

change in binding mode or receptor conformation to accommodate compound **6**. Attempts to determine a crystal structure of BACE-1 complexed with **6** were not successful, possibly because of its low solubility in the buffer used for soaking of BACE-1 crystals.

The modifications of the initial tyramine fragment lead to significant improvement of ligand binding affinity while the ligand efficacy of the compounds was kept around 0.35. This is remarkable for the drug target BACE-1 which is considered to exhibit low chemical tractability and supports the view that the S1 pocket is a hot spot for binding capability. In order to assess if the molecules show functional activity, an enzyme inhibition analysis was performed to complement the characterization of the compounds. For all but molecule **2** inhibition of enzymatic activity could be detected confirming the results by surface plasmon resonance. However, due to the high ligand concentrations required to measure weak inhibition ($IC_{50} > 100\ \mu M$), IC_{50} values could not be calculated with confidence and therefore no correlation between K_D and IC_{50} values could be established. This illustrates the superiority of biophysical binding assays like surface plasmon resonance over enzymatic assays for the identification of weakly binding fragments.

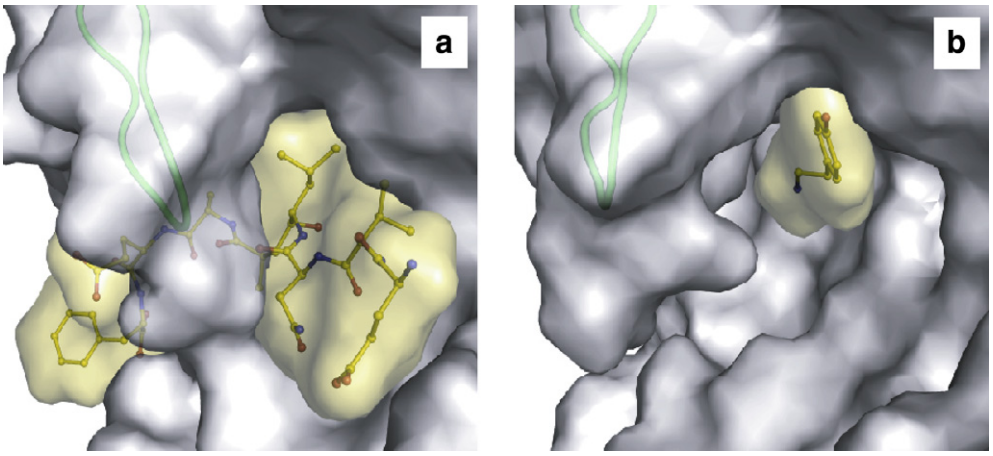


Figure 3. Surface representation of (a) OM99-2[3] and (b) tyramine **1** bound to the BACE-1 active site. The flap indicated in green adopts the closed conformation in (a) and the open conformation in (b).

Fragment screening efforts reveal that the tyrosine metabolite tyramine and derivatives thereof bind to the active site of BACE-1. Due to their low molecular weight and high ligand efficiency these compounds serve as ideal starting points for the design of BACE-1 inhibitors with CNS pharmacological activity. In addition, the obtained structural information can be used for morphing or scaffold hopping approaches to improve other chemical lead series.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.01.032.

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9. Surface Plasmon Resonance measurements were performed on a Biacore S51 instrument. BACE-1 was immobilized (~12,000 RU) by standard amine coupling chemistry on a CM-5 sensor. Binding experiments were performed using acetate buffer (50 mM, pH 4.6, 150 mM NaCl, 3 mM EDTA, 0.005% P20, and 4% DMSO) as the running buffer. Compounds were dissolved in 100 mM DMSO and subsequently diluted into acetate buffer in order to adjust the final DMSO content and the concentration of the respective compound. Hits were further investigated for specific binding to the active site of the enzyme by competition experiments using a high affinity (K_D = 40 nM) inhibitor of BACE-1 derived from the substrate (pGlu-Val-Asn-statin-Val-Ala-Glu-Phen-am).
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13. The K246A surface mutant variant of BACE-1 ectodomain (20 mg/ml) was crystallized by vapor diffusion in hanging drops at 20 °C by mixing 1.5 µl protein solution with 0.5 µl of 2.5 M sodium formate, 100 mM Hepes (pH 7.0). Crystals of space group P6122 with unit cell dimensions $a = b = 103$ Å, $c = 169$ Å grew to a maximum size of $100 \times 100 \times 400$ µm^3. Apo crystals were soaked for 8–16 h in a buffer containing 30–100 mM compound, 2.5 M sodium formate, 0.1 M sodium acetate (pH 4.5), and 10% DMSO. X-ray diffraction data were collected at beamline X06SA of the Swiss Light Source (Paul Scherrer Institut, Villigen, Switzerland). The crystal structure of the K246A variant protein was solved by molecular replacement using coordinates of an unpublished BACE-1 ectodomain structure as search template.